Platelet Membrane Activation Markers Are Predictive for Increased Risk of Acute Ischemic Events After PTCA

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Background. We wished to investigate whether platelet activation is related to the clinical outcome during the 24 hours immediately after elective percutaneous transluminal coronary angioplasty (PTCA).

Methods and Results. In 102 patients with high-grade coronary stenosis admitted for elective PTCA, preprocedural platelet activation was characterized by flow cytometric measurement of the proteins CD62, CD63, and thrombospondin expressed on the platelet surface membrane. The prevalence of acute ischemic events during the 24 hours immediately after the procedure was then related to the pre-PTCA platelet activation status. Fifty-six patients were classified as "nonactivated," whereas 46 patients showed an increased percentage of activated platelets. Two patients developed acute occlusion (1.96%) and four patients high-grade restenosis (3.92%), as confirmed by second-look coronary angiography. All events occurred in patients classified as "activated" (six of 46, or 13%). None of these patients received β-blocker medication, which was associated with lower expression of platelet membrane activation markers. In the nonactivated patient group, no clinical events were found (0 of 56, or 0%). This difference in prevalence is significant (p=0.007).

Conclusions. We conclude that analysis of platelet membrane activation markers may help to predict an increased risk of acute ischemic events after angioplasty. (Circulation 1993;88:37-42)

KEY WORDS • angioplasty • platelets • ischemia

Coronary heart disease is associated with intravascular platelet activation.1-3 Percutaneous transluminal coronary angioplasty (PTCA) is one established therapy for patients with localized coronary stenosis as an alternative to bypass surgery. The success of this intravascular dilatation technique is limited either by reocclusion or high-grade restenosis occurring early after dilatation and by long-term restenosis. Complications may be caused by a preexistent prethrombotic state or by activation of the hemostatic system by the PTCA maneuver itself. There is still a diagnostic gap, because no predictive laboratory test has been established to identify patients at increased risk. With single-platelet flow cytometry (SFFC), it is possible to detect in vivo platelet activation in individual patients after staining platelet samples for thrombospondin-, CD62-, and CD63-related antigens that are exposed after activation.4-5 With this technique, this study was intended to clarify whether platelet activation is related to the early clinical outcome after elective PTCA.

Methods

Subjects and Study Design

Patients with proven coronary heart disease (CHD, n=102) admitted to the University Clinic of Dusseldorf for elective PTCA were consecutively recruited when a high-grade coronary vessel stenosis of ≥85% was confirmed by visual analysis of the recent angiographic record. None of the recruited patients showed unstable angina. Patients with acute infarctions, hematological diseases, and diabetes mellitus were excluded. No exclusions were made for medication. Except for those with contraindications, all patients received acetysalicylic acid (ASA) and calcium channel blockers at least 1 day before PTCA. Basic clinical characteristics and pretreatment are shown in Table 1.

On the day of PTCA, a blood sample was taken for platelet activation marker analysis, and the patient was classified as having an "activated" or "nonactivated" platelet status. The classification result was not for-
TABLE 1. Clinical Characteristics of Patients Before Percutaneous Transluminal Coronary Angioplasty, Classified to Have an 'Activated' or 'Nonactivated' Platelet Status

<table>
<thead>
<tr>
<th>Anthropometric indexes</th>
<th>Platelet activation status</th>
</tr>
</thead>
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<tr>
<td>Gender (M/F)</td>
<td>Cohort (n=102)</td>
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<tr>
<td>Age (years)</td>
<td>84/18</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>55.3±9.8</td>
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<td>Laboratory parameters</td>
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<tr>
<td>Cholesterol (mg/dL)</td>
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</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>185.3±106.5</td>
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<tr>
<td>Fibrinogen (mg/dL)</td>
<td>318.7±85.1</td>
</tr>
<tr>
<td>Quick's test value (%)</td>
<td>94.6±18.7</td>
</tr>
<tr>
<td>PTT (seconds)</td>
<td>39.2±18.6</td>
</tr>
<tr>
<td>Thrombin time (seconds)</td>
<td>25.5±28.1</td>
</tr>
<tr>
<td>Platelet count (x10^9/μL)</td>
<td>270.7±74.5</td>
</tr>
</tbody>
</table>

Risk factors
Smoking (%) | 74 (75.5) | 39 (69.6) | 35 (76.1) |
Hypertension (%) | 59 (57.8) | 33 (58.9) | 26 (56.5) |

Chronic pretreatment
ASA | 88 (86.2) | 46 (82.1) | 42 (91.3) |
Nitrates | 97 (95.1) | 54 (96.4) | 43 (93.5) |
Calcium antagonists | 91 (89.2) | 52 (92.8) | 39 (84.8) |
β-Blockers | 62 (60.8) | 40 (71.4) | 22 (47.8)* |
Heparin | 15 (14.7) | 8 (14.2) | 7 (15.2) |

Coronary vessel stenosis
One-vessel disease | 55 (57.8) | 27 (48.2) | 28 (60.9) |
Two-vessel disease | 28 (27.5) | 19 (33.9) | 9 (19.6) |
Three-vessel disease | 19 (18.6) | 10 (17.8) | 9 (19.6) |

PTCA characteristics
Dilated vessel
LAD | 47 (46.1) | 24 (42.9) | 24 (50.0) |
RCA | 36 (35.3) | 21 (37.5) | 15 (32.6) |
RCx | 19 (18.6) | 11 (19.6) | 8 (17.4) |
Distal stenosis of the dilated vessel | 15 (14.7) | 7 (12.5) | 8 (17.4) |
Endothelial dissections | 19 (18.6) | 10 (17.9) | 9 (19.6) |
No. of dilations | 3.6±2.1 | 3.6±2 | 3.7±2.1 |
Max duration of dilatations (seconds) | 77.8±23.9 | 80.6±25.3 | 74±21.7 |
Max pressure of dilatations (atm) | 7.6±1.9 | 7.7±1.7 | 7.5±2.2 |

PTT, partial thromboplastin time; ASA, acetylsalicylic acid; LAD, left anterior descending coronary artery; RCA, right coronary artery; RCx, ramus circumflexus, or left posterior descendent artery (LPD); Max, maximum. Values are mean±SD or n (%).
*p<0.05, significant difference between subgroups.

The patient was then monitored for 24 hours.

Clinical End Points

The end point "acute ischemic event" was defined as rapid onset of prolonged angina similar to chest pain during procedural balloon insufflation and new ECG signs of myocardial ischemia10 (ST segment elevation >0.2 mV in at least two leads). In these cases, a second-look coronary angiography was performed (according to Reference 11).

Acute ischemic events were attributed to occlusion or high-grade restenosis. Occlusion was defined as acute perfusion stop, and high-grade restenosis was defined as relapse of the vessel stenosis to or above pretreatment...
values (≥85% luminal narrowing). Without clinical symptoms, ECG signs of acute ischemic events, or total creatine kinase elevation greater than twice the upper limit of normal values during the 24 hours immediately after the procedure, PTCA success was assumed to be stable.

Blood Sampling

After the patient fasted overnight, venous blood was taken without tourniquet from resting subjects directly into platelet-stabilizing reagent (134 mmol/L EDTA, 0.7% hydroxychloroquine sulfate, 20 units/mL heparin; ratio, 9 volumes blood plus 1 volume reagent). Thereafter, the sample was immediately fixed with paraformaldehyde13 (0.5%, pH=7.4).

Antibodies

The monoclonal antibodies P10, 2.17, and 2.28 have been described previously.5-10 Antibody P10 reacts with thrombospondin, and 2.28 reacts with the CD63-related antigen, a 53-kd lysosomal integral membrane protein. Antibody 2.17 reacts with the CD62-related antigen, a 140-kd component of the internal α-granule membrane. These monoclonal antibodies react specifically with activated platelets.4-7,9-12

Flow Cytometry

The SPFC assay was performed according to References 4, 13, and 14. The fixed platelets were simultaneously stained with P10, 2.17, and 2.28 monoclonal antibodies and labeled with F(ab)2-FITC fragments. Fluorescence-activated cell sorter analysis (Facstar, Becton Dickinson, San Jose, Calif) was performed immediately after staining. The result is expressed as percentage of specific fluorescence-positive platelets. The overall interassay variance was appraised on the basis of the mean coefficient of variation from all measurements comprising three independent samples each: 17% (range, <5% marker-positive platelets), 10% (range, 5-10% marker-positive platelets), and 8% (range, >10% marker-positive platelets).

Platelet Activation

The patients were classified to have an activated platelet status when exceeding the mean±3 SD normal range for the percentage of marker-positive platelets in any marker (thrombospondin >5.6%, CD62 >5.1%, CD63 >3.4%).

Other Laboratory Investigations

Platelet counts were determined by a Coulter Counter (Hialeah, Fla). All other parameters documented were measured with routine methods.

Statistical Analysis

Statistics are presented according to References 15 and 16. Mean±SD was used for descriptive statistics. Contingency tables were used to relate clinical end points with the pre-PTCA platelet activation status. Groups were compared by χ² statistics or by Fisher's exact method as appropriate. The one-tailed significance threshold was set at p<0.05.

Results

All 102 elective PTCA maneuvers were completed with successful dilatation of the stenosed vessel on the basis of the coronary angiography at the end of PTCA (residual stenosis as evaluated by visual analysis was <50% of the native vessel diameter in all cases). Maintenance of PTCA success over a period of 24 hours was achieved in 96 patients (94.1%) and confirmed by normal treadmill ECG after 48 hours. Within the first 24 hours after PTCA, symptoms of acute ischemic events developed in six patients (5.9%) in whom a second-look coronary angiography was performed immediately after onset of symptoms. Occlusion was found in two patients (1.96%, TIMI grade 0) and high-grade restenosis in four (3.92%, TIMI grade 1-2). There was no case of death or emergency surgery in this series of patients. Clinical data and procedural characteristics are summarized in Table 2.

All patients had generalized caliber irregularities of the dilated vessel, but only a minor fraction showed significant stenosis distal to the dilated area (Table 1). None of these developed acute ischemic episodes. During the PTCA procedure, one patient developed intermittent vasospastic vessel occlusion, and mural thrombosis was suspected in another patient (both from the activated subgroup), but none of these developed ischemic episodes. Visible endothelial dissection was observed in both groups, activated and nonactivated patients, to a similar extent (Table 1) and occurred in three of those six patients with verified post-PTCA acute ischemic events (Table 2).

A nonactivated platelet status before PTCA was found in 56 patients (54.9%), and 46 patients (45.1%) were classified as activated (Figure 1). All six postprocedural ischemic events occurred in patients from the activated group showing the highest levels of marker-positive platelets (Table 2; prevalence, six of 46, or 13%). However, no ischemic event was found in the nonactivated group (prevalence, 0 of 56, or 0%; Figure 2). The difference between the two patient groups is significant (p=0.007).

Since β-blocker medication was apparently more frequent in the nonactivated group, the statistical analysis was also performed in the subset of patients who received no β-blockers (n=40). In 24 of these patients, the platelet status was activated and the prevalence of post-PTCA ischemic events increased to 25% in this group (n=6), whereas no ischemic event was found in the patients with nonactivated platelets (p=0.035; Figure 2B). Moreover, there were more activated platelets in patients without than in patients with β-blocker medication (thrombospondin, 7.6±6.2% versus 4.2±3.2%, p<0.01; CD62, 6.3±6.1% versus 4.1±3.2%, p<0.05; CD63, 4.4±5.9% versus 3.5±4.5%, NS).

Discussion

This prospective study provides evidence that platelet membrane activation markers are associated with the early clinical outcome of elective PTCA. The overall prevalence of early post-PTCA ischemic events (5.9%) was in line with recent reports but reflected exclusively patients with activated platelets.10,17-23 Known risk factors associated with periprocedural occlusion (e.g., multivessel disease, residual stenosis, endothelial dissec-
tion, or PTCA characteristics) were equally distributed between the study subgroups and those patients who subsequently suffered from documented occlusion and high-grade restenosis10,17,24,25 (Tables 1 and 2). However, there was a dominance of dilated left anterior descending coronary artery (LAD) among those patients with post-PTCA ischemic episodes compared with the frequency of LAD dilatation in the cohort.

It must be admitted that the study design does not rule out the possibility that clinically silent vessel restenosis/occlusion occurred, because second-look angiography after 24 hours was not performed in all cases. However, this seems improbable because none of the asymptomatic patients developed ECG changes or a significant increase in creatine kinase. Conversely, the positive angiographic findings in all six cases indicate that cases of restenosis/occlusion are detected by the combination of clinical and ECG signs of myocardial ischemia. The precise detection rate, however, remains unclear.

![Platelet Activation Status](https://circ.ahajournals.org/doi/figure/1)

**FIGURE 1.** Bar chart showing expression of the three activation-dependent antigens (given as mean [%] percentage of “marker-positive” platelets±SD) in the cohort and in the “nonactivated” and “activated” patient subset.

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### TABLE 2. Clinical Characteristics of Six Patients With Acute Post-Percutaneous Transluminal Coronary Angioplasty Ischemic Events

<table>
<thead>
<tr>
<th>Patient</th>
<th>IO</th>
<th>2R</th>
<th>3R</th>
<th>4R</th>
<th>5O</th>
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<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>P.10 (Thrombospondin) (%)</td>
<td>22.1 †</td>
<td>4.7 †</td>
<td>7.7 †</td>
<td>8.9 †</td>
<td>21.6 †</td>
<td>6.7 †</td>
<td>12±7.8</td>
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<tr>
<td>2.17 (CD62) (%)</td>
<td>15.6 †</td>
<td>5.2 †</td>
<td>7.7 †</td>
<td>6.3 †</td>
<td>23 †</td>
<td>4.5</td>
<td>10.4±7.4</td>
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<td>2.28 (CD63) (%)</td>
<td>1.6</td>
<td>1.8</td>
<td>1.5</td>
<td>2.5</td>
<td>20.3 †</td>
<td>19.1 †</td>
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<td>Platelet count (µL⁻¹)</td>
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<td>201 000</td>
<td>124 000</td>
<td>262 000</td>
<td>259 000</td>
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<td>84</td>
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<td>221</td>
<td>232</td>
<td>98</td>
<td>109</td>
<td>161.2±63.8</td>
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<td>Anatomy of coronary vessel stenosis</td>
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<td>5</td>
<td>4</td>
<td>2</td>
<td>3.7±1.4</td>
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<td>Maximal duration (seconds)</td>
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<td>90</td>
<td>120</td>
<td>75</td>
<td>60</td>
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<tr>
<td>Maximal pressure (atm)</td>
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<td>6</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>7.7±2.6</td>
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</table>

O, occlusion; R, restenosis; ASA, acetylsalicylic acid; †, increase; PTCA, percutaneous transluminal coronary angioplasty; RCx, ramus circumflexus, or left posterior descending artery (LPD); LAD, left anterior descending coronary artery; RCA, right coronary artery.
Platelet activation has been shown to initiate unstable angina preceding acute myocardial infarction.1-3 A prethrombotic state may also contribute to thrombotic occlusions induced by angioplasty.18,26 An increased percentage of circulating platelets expressing thrombospondin-1, CD62L, and CD63-related antigens on the surface membrane was assumed to reflect in vivo activation of platelets.6,9,27,29 Such an activation of the platelet system has been shown for various disease states, e.g., cardiopulmonary bypass or respiratory distress,6,9,27,30 and the detection of increased platelet activation markers might be used to identify patients with a thrombotic diathesis.4,9,14,28-30 Within this group of patients with coronary heart disease, we found increased platelet activation in 45.1%. Highest values of activated platelets were present in those six patients with consecutive acute post-PTCA ischemic events angiographically classified as reocclusion (n=2) as well as restenosis (n=4). However, peak values of platelets positive for at least two of the three tested markers were found in the two cases of reocclusion despite similar treatment with a combination of drugs with approved antiplatelet properties. From the baseline evaluation of the study patients, it cannot be decided whether the pretreatment even decreased the level of platelet activation from formerly higher to still elevated values or whether it was just ineffective. In patients taking β-blockers, platelet activation was less frequent than in those without β-blocker medication. This may result from the membrane-stabilizing antiplatelet potential of these drugs.31,32 Although 62% of the cohort received β-blockers, none of the patients with acute post-PTCA ischemic events received these drugs. Therefore, pretreatment with ASA, calcium channel blockers, nitrates, and heparin seems not to be responsible for the observed difference in acute post-PTCA ischemic events,28,33 whereas β-blockers might reduce the risk of such complications by attenuating early platelet activation.

Our study aimed to show an association between platelet activation and post-PTCA ischemic events. Although not valid for definitive evaluation because of the small numbers of primary study end points, the data base presented allows an estimate of the expected sensitivity and specificity of the activation marker test with regard to acute post-PTCA ischemic events (Figure 2A): sensitivity tends to be high [a/(a+b)=6/(6+0)=100%] at lower specificity [d/(c+d)=56/(40+56)=58.3%]. In view of the relevance of acute coronary ischemic events after PTCA for the clinical prognosis of the patients, this configuration may be acceptable (Reference 16, chapter 7). However, even when the estimate of sensitivity is lowered to 90%, the predictive value of the positive test (activated platelet status) for acute ischemic events accounts for 12%, with an overall prevalence of 6% [(a+b)/(a+b+c+d)], according to the theory of Bayes.16 This indicates that independent of potentially predictive angiographic variables, patients with an activated platelet status have an estimated twofold higher prevalence (12% instead of 6%) of acute post-PTCA ischemic events in this study. Furthermore, the relative disease risk of patients with a positive test (activated platelet status) accounts for 12 compared with a negative test result.

Several conclusions may be drawn. 1) Platelet activation marker analysis provides predictive information on the incidence of occlusive vascular complications after angioplasty. 2) In view of the significance of acute post-PTCA occlusion for morbidity and mortality,10,17-20,22,23,34 testing of all patients before an elective PTCA for the purpose of a thrombotic risk assessment might be considered. However, such a general recommendation can be made only after further confirmation of the study results presented.

Acknowledgments

We gratefully acknowledge the critical discussion of P. Roesen, Duesseldorf, FRG, and the supply of specified monoclonal antibody material by M. Metzelaar, Utrecht, The Netherlands.

References


Platelet membrane activation markers are predictive for increased risk of acute ischemic events after PTCA.

Circulation. 1993;88:37-42
doi: 10.1161/01.CIR.88.1.37

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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